

AMENDMENT

IN THE SPECIFICATION:

Paragraph beginning at line 19 of page 7 has been amended as follows:

Figures 2A-2C show[[s]] Northern blot analyses of *Evi27* expression in murine tissues and cancer cell lines. Multiple tissue Northern blots of normal adult (**Figure 2A**), BXH2 leukemic cell lines (**Figure 2B**), and various hematopoietic cell lines (**Figure 2C**): M1, myeloid leukemia, WEHI3B, monocyte; HYB. 548, B-cell hybridoma; WEHI231, preB-cell lymphoma; R1.1, lymphocytic thymoma; EL4, thymoma, P815, mastocytoma. The blots were also hybridized with a GAPDH or β -actin probe to control for RNA loading. The size in kilobases (kb) of molecular weight markers is shown on the left of each panel. To the right of each panel are the sizes of the *Evi27* transcripts observed.

Paragraph beginning at line 10 of page 8 has been amended as follows:

Figures 3A-3C show[[s]] Northern blot analyses of *EVI27* expression in human tissues and cancer cell lines. Multiple tissue Northern blots of normal adult (**Figure 3A**), immune tissues (**Figure 3B**) and cancer cell lines (**Figure 3C**) hybridized with an EST specific for the human *EVI27* gene. The human cancer cell lines are HL60, promyelocytic leukemia; HeLa, cervical carcinoma; K562, chronic myelogenous leukemia; MOLT4, T-lymphoblastic leukemia; Raji, Burkitt's lymphoma; SW480, colon adenocarcinoma; A549, lung carcinoma; G361, melanoma. The blots were also hybridized with a β -actin probe to control for RNA

loading. The size in kb of molecular weight markers is shown on the left of each panel.

Paragraph beginning at line 15 of page 9 has been amended as follows:

Figures 6A-6B show an alignment of the human EVI27 and IL-17R proteins. Conserved amino acids are boxed with amino acid identities noted in bold. Gaps created in the sequences to optimize alignments are represented by dashes. Amino acid positions are indicated to the right and left of the sequence.

Paragraph beginning at line 7 of page 10 has been amended as follows:

Figures 8A-8D show immunofluorescence and Northern blot analysis of *Evi27* expression in murine cell line. The 2.7 kb cDNA coding for the 55 kD isoform of the membrane bound form of the murine *Evi27* gene was cloned into a eukaryotic expression plasmid vector and transfected into the murine myeloid leukemia cell line 32D. A cell line was established by limiting dilution and called 32D*Evi27A*. **Figure 8A** shows a Northern blot hybridization of poly-A mRNA from 32D and 32D/*Evi27A* stable transfectants. Note the abundant expression of the transgene in the transfectant and the absence of expression in the parental line. The blot was also hybridized with a β -actin probe to control for RNA loading. The size in kb of molecular weight markers is shown on the left of each panel. **Figure 8B** shows Western blot analysis of same cell lines with affinity purified anti-*Evi27* antisera. The transfectant shows overexpression of a 55 kD protein as expected from predicted mRNA translation of the 2.7 kb *Evi27* cDNA. Markers in kD are to the left. **Figure 8C** shows immunofluorescence staining of the *Evi27* protein (red) in the myeloid cell line 32D (top panel) and 32D cells transfected with an

Evi27 cDNA expression construct (lower panel). Note the light staining in the parental line and abundant staining in the transfectant (left panels). Nuclei are stained blue with DAPI. Cells were also stained with *Evi27* antibody preincubated with *Evi27* peptide (right panels). Note that no red staining is evident, demonstrating specificity of the antisera. **Figure 8D** shows cell surface expression of *Evi27* by flow activated cell sorting analysis (red: anti-*Evi27*; blue: anti-*Evi27*+peptide; green: IgG control).

Paragraph beginning at line 20 of page 12 has been amended as follows:

Figures 12A-12B show[[s]] Western blot analysis of *Evi27* protein expression in BXH2 leukemia cell lines, EL4 and R1.1 T-cell lines, and the WEHI231 B-cell line. 10 µg of protein from cell lysates was run in tandem and hybridized with *Evi27* antibody either without (**Figure 12A**) or with (**Figure 12B**) preincubation with *Evi27* peptide. Note the absence or reduction of specific bands in panel (**Figure 12B**) compared to (**Figure 12A**). Molecular weight standards in kD are indicated to the left. The *Evi27* isoforms and sizes are indicated to the right of panel A.

Paragraph beginning at line 1 of page 47 has been amended as follows:

Northern blot analysis of mouse tissue using both exons as probes confirmed this prediction and showed that the *Evi27* hybridization pattern is complex. Six different *Evi27* transcripts of approximately 4.4, 4.2, 2.3, 1.9, 1.3, and 1.1 kb in size could be detected on northern blots (**Figures 2A-2C**). In adult tissues the expression was seen in liver and testes where the 2.3 and 1.9 kb transcripts predominated. Expression of the 4.2 transcript was also seen in liver and testes. In addition, low levels of the 2.3 and 1.9 kb transcripts were seen in kidney (**Figure 2A**). With long exposure, the lung showed expression of a 2.1 kb transcript, while the

heart showed expression of the 1.3 kb transcript. No expression was seen in skeletal muscle, brain, or spleen.

Paragraph beginning at line 9 of page 48 has been amended as follows:

In humans, two *EVI27* transcripts, 1.9 kb and 2.7 kb in size, were detected (Figures 3A-3C). Human *EVI27* expression is therefore considerably less complex than in mouse. The highest *EVI27* expression was seen in kidney (Figure 3A), while in the mouse, *Evi27* was expressed at low levels in the kidney. Moderate expression was also observed in the brain, liver, and testes with low to undetectable expression in lung. No *EVI27* expression was seen in heart, placenta, and skeletal muscle (Figure 3A) or in immune tissues such as spleen, lymph nodes, thymus, peripheral blood lymphocytes, or bone marrow (Figure 3B). High *EVI27* expression was, however, observed in the fetal liver.

Paragraph beginning at line 19 of page 52 has been amended as follows:

Amino acid sequence comparisons showed that *Evi27* has significant homology throughout its coding region to the human and mouse *IL-17 receptor (IL-17R)* (E value = 2.5×10^{-29}) (Figures 6A-6B). The position of the transmembrane domain with respect to the amino terminus is essentially the same in the two proteins. However, the cytoplasmic tail of the *IL-17R* is nearly 304 amino acids longer than that of the *Evi27*. Although *Evi27* is predicted to encode both membrane bound and soluble forms, *IL-17R* is not known to encode a soluble form.

Paragraph beginning at line 13 of page 53 has been amended as follows:

The human *EVI27* gene was mapped to chromosome 3p21 by fluorescence *in situ* hybridization of high-resolution G-banded

chromosome (Figures 7A-7B). These results are consistent with the mouse mapping data, which localize the mouse gene to chromosome 14 in a region of human 3p21 homology (Copeland et al., 1995). They also confirm that the human gene maps in a region of the human genome that is frequently rearranged in human myeloid leukemia.